REMARKS

Claims 1 – 5, 7, 8, 14 and 45 - 47 are pending in the application. Claim 45 has been amended. Claims 6, 9 – 13, 15 - 44 have been canceled as being directed to non-elected inventions. No new claims have been added. No new matter has been added by virtue of the amendments, support being found in the specification and in the claims as originally filed.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Claim Rejections- 35 U.S.C. § 112, second paragraph

The Examiner has maintained the rejection of claims 45 and 47 under 35 USC §112, second paragraph as being indefinite. Applicants respectfully traverse the rejection.

The Examiner indicates that the specification "fails to provide sufficient support for 'one or more of a method selected from nuclease treatment, mechanical shearing chemical treatment or radiation treatment.'" (Office Action, p.2 – 3). The Examiner argues that "there is no support in the specification for the combination of one or more method selected from nuclease treatment, mechanical shearing, chemical treatment and radiation treatment." (Office Action, p.3). Applicants have amended the claims and respectfully disagree.

Claim 45 depends from claim 1, and recites that the inserting randomly of an insertion nucleic acid sequence into an acceptor nucleic acid sequence is carried out by a method selected from: nuclease treatment, mechanical shearing, chemical treatment or radiation treatment.

Applicants submit that the amendments to the claims clarify that a method selected from nuclease treatment, mechanical shearing, chemical treatment or radiation treatment refer to methods that are used in the random insertion of an

insertion nucleic acid sequence into an acceptor nucleic acid sequence as set forth in the claims. Applicants direct the Examiner to the specification at paragraph [0130], where a number of different strategies can be used to create the fusion molecules of the instant invention, including nuclease treatment, mechanical shearing, chemical treatment or radiation treatment. Applicants refer the Examiner to paragraph [0140] of the application that teaches "it should be obvious to those of skill in the art that any method of introducing breaks into a DNA molecule can be used (e.g., such as digestion by mung bean nucleases, endonucleases, restriction enzymes, exposure to chemical agents, irradiation, and/or mechanical shearing)."

Applicants respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 112, first paragraph

The Examiner has maintained the rejection of claims 45 - 47 under 35 USC §112, first paragraph, for allegedly failing to comply with the enablement requirement. The Examiner argues that "the specification, while being enabling for treating a nucleic acid with nuclease treatment, mechanical shearing, chemicals or radiation for the claimed method in vitro, does not reasonably provide enablement for treating a nucleic acid with nuclease, mechanical shearing, chemicals or radiation for the claimed method in vivo, or for treating any molecule other than nucleic acid. Applicants respectfully disagree.

As amended, the claims recite inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence carried out by a method selected from nuclease treatment, mechanical shearing, chemical treatment or radiation treatment.

Applicants submit that the claims, as amended, are fully enabled, and respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 102 (b)

The Examiner has maintained the rejection of claims 1 – 5, 7, 8 and 14 under 35 USC 102(b) as being anticipated by Lacatena et al. (PNAS, Vol. 91, pp.10521 – 10525. 1994). Applicants respectfully traverse the rejection.

As set forth above, the claims recite a method for assembling a modulatable molecule, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state, thereby generating a nucleic acid fusion molecule; and selecting a nucleic acid molecule that encodes a polypeptide wherein the state of the polypeptide encoded by the acceptor nucleic acid is coupled to the state of the polypeptide encoded by the insertion nucleic acid, or the state of the polypeptide encoded by the insertion nucleic acid is coupled to the state of the polypeptide encoded by the acceptor nucleic acid.

To anticipate a claim, each and every element of the claim must be found in a single reference. This is discussed in the Manual of Patent Examining Procedure § 2131:

> "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the . . . claim." Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989). The elements must be arranged as required by the claim, but this is not an ipsissimis verbis test, i.e., identity of terminology is not required. In re Bond, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990).

The Lacatena reference does not teach or suggest all the limitations of the instant claims. In particular, the Lacatena reference does not teach or suggest assembling **a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, wherein the

insertion nucleic acid sequence and the acceptor nucleic acid sequence **each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

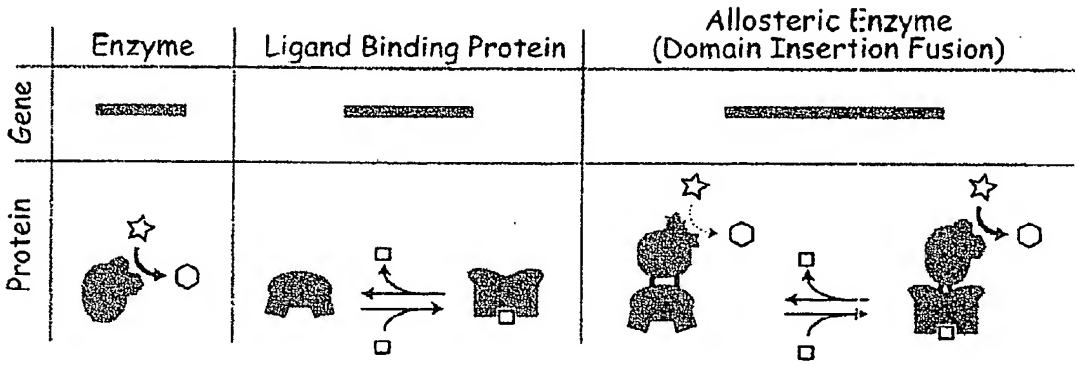
The Lacatena reference simply teaches a fusion molecule between TnphoA and hubeta2AR where, as pointed out by the Examiner "PhoA can be considered as an insertion sequence and the hubeta2AR protein can be considered as an acceptor sequence (and) PhoA encodes bacterial alkaline phosphatase and hubeta AR2 encodes human beta2-adrenergic receptor." (Office Action p, 5). Lacatena et al. "analyze the assembly of the (human β2 adrenergic receptor) membrane protein in E-coli by using a set of huβ2 AR-PhoA fusions in order to determine the topology in the bacterial membrane and the requirements for the correct insertion into the membrane." (page 10521). Lacatena use the PhoA activity of the fusion proteins to determine if the huβ2 AR-PhoA fusions have acquired the correct topology, where "high activity is observed when the phosphatase domain is located on the outside of the cell membrane, whereas fusions in which phosphatase sequences are located on the inside of the membrane yield low activity." (p.10523). As pointed out by the Examiner "Lacatena teaches assaying the alkaline phosphatase activity of the fusion protein." (Office Action, p.5). The fusion taught by Lacatena retains the function of the inserted protein. Accordingly, the Lacatena reference simply teaches a fusion molecule, where the levels of PhoA activity is a measure of huβ2 AR topology.

The present invention teaches that both the insertion sequence and acceptor sequence are capable of existing in at least two states and the state of the insertion sequence is coupled to the state of the acceptor sequence upon fusion, **such that a change in state in either the insertion sequence or acceptor sequence will result in a change in state of respective other portion of the fusion**. (see, e.g. page 17, line 29). As taught by the specification at page 13, beginning at line 16, a "'state of a molecule' or a "state of a portion of a molecule" can be a conformation, binding affinity, or activity (e.g., including, but not limited to, ability to catalyze a substrate; ability to emit light, transfer electrons, transport or localize a molecule, modulating transcription, translation, replication, supercoiling, and the like)." The specification describes "an insertion sequence" at page 14, line 13, as referring to "a polymeric sequence which is

contained within another polymeric sequence (e.g., an "acceptor sequence") and which conditionally alters the state of the other polymeric sequence." Further, as taught on page 13, beginning at line 29, "'coupled' refers to **a state which is dependent on another state such that a measurable change in the other state is observed.**"

First, Applicants point out that the present invention is based on the idea that the acceptor sequence and the insertion sequence exist in **two separate states**, and the separate states are coupled in a fusion molecule. For example, when an enzyme (insertion) and a ligand binding protein (acceptor) each encode a state, the state of the polypeptide encoded by the ligand binding protein (acceptor) is coupled to the state of the polypeptide encoded by the enzyme (insertion), or the state of the polypeptide encoded by the enzyme (insertion) nucleic acid is coupled to the state of the polypeptide encoded by the ligand binding protein (acceptor) nucleic acid. Applicants refer to the example illustrated in the schematic below, also described in Example 1 of in the specification (page 52). As described on page 52 beginning at line 13, "a model system consisting of E. coli maltose binding protein ("MBP") as the acceptor polypeptide sequence and the penicillin-hydrolyzing enzyme TEM1 β-lactamase as the insertion polypeptide sequence was chosen to test the combinatorial domain insertion strategy for coupling the two proteins' function. The desired property of the model switch is the ability to modulate β-lactamase activity through changes in maltose concentration (i.e., the switch molecule or fusion protein would behave as an allosteric enzyme). The

schematic below simplifies this model switch:



Further, Figure 3, shown below, also illustrates methods of using molecular switches according to the invention as claimed. FIG. 3A shows regulation of gene transcription using a fusion molecule. FIG. 3B shows modulation of a cell signaling pathway. FIG. 3C shows drug delivery mediated by a fusion molecule to a cell expressing a marker of a pathology. FIG. 3D shows the use of fusion molecules for drug transport to an intracellular compartment. FIG. 3E shows delivery of a conditionally toxic fusion molecule to a cell. FIG. 3F shows the use of a fusion molecule for metabolic engineering. FIG. 3G shows a fusion molecule according to one aspect of the invention which functions as a biosensor

FIG. 3A

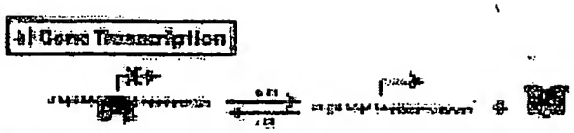


FIG. 3B

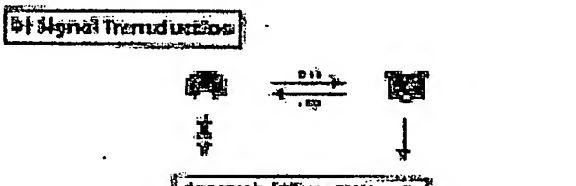


FIG. 3C

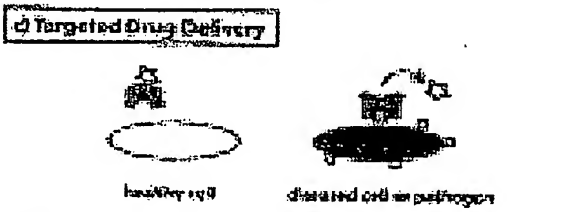


FIG. 3D

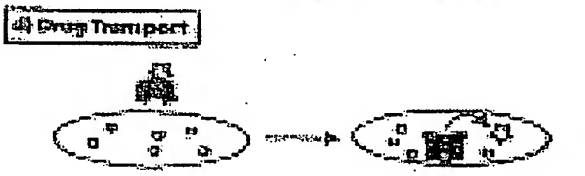


FIG. 3E

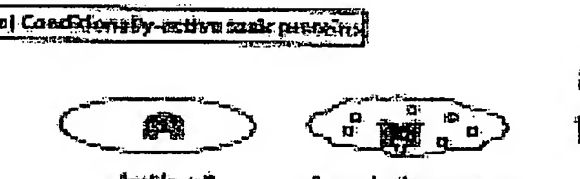


FIG. 3F

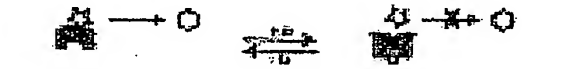
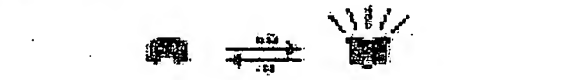


FIG. 3G



As shown in the examples above and taught throughout the specification, both the insertion sequence and acceptor sequence are capable of existing in at least two states and the state of the insertion sequence is coupled to the state of the acceptor sequence upon fusion, such that a change in state in either the insertion sequence or acceptor sequence will result in a change in state of other portions of the fusion protein.

The Lacatena reference does not teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence **each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 102 (e)

The Examiner has maintained the rejection of claims 1 – 5, 7, 8 and 14 under 35 USC 102(e) as being anticipated by Anderson et al. (US Patent No. 6,596,485). Applicants respectfully traverse the rejection.

The claims were set forth above.

The Examiner argues that the Anderson reference "teaches fusing random peptide into GFP to generate GFP fusion protein via insertion of nucleic acid (and) (t)he random peptide is fused to an internal position of the GFP fusion protein via insertion of nucleic acid." (Office Action, p.6). The Examiner argues that "(g)eneration of the GFP fusion protein constitutes insertin of an insertion sequence into an acceptor sequence and said insertion couples the state of the insertion sequence to the state of the acceptor sequence (and) (t)he peptide can be considered as an insertion sequence and the GFO can be considered as an acceptor sequence." (Office Action, p.6).

As Applicants have pointed out before; the Anderson reference provides fusions of green fluorescent protein (GFP) and random peptides, and teaches a fusion nucleic acid comprising a first nucleic acid encoding a GFP scaffold protein; a second nucleic acid encoding a linker fused to the C-terminus of the scaffold protein and a third nucleic acid encoding a random peptide fused to the C-terminus of the linker. The GFP and the second and third nucleic acids taught by Anderson **do not each comprise a state** such

that the state of one is coupled to the state of another such that a measurable change in the other state is observed.

The Anderson reference is directed to fusion proteins comprising a random peptide fused to GFP. Anderson describe fusions of GFP such that the structure of the GFP is not significantly perturbed and the peptide is metabolically conformationally stabilized. According to Anderson, "the GFP is fused to a random peptide to form a fusion polypeptide. By "fused" or "operably linked" herein is meant that the random peptide, as defined below, and the GFP, are linked together, in such a manner as to minimize the disruption to the stability of the GFP structure (i.e. it can retain fluorescence, as outlined herein) or maintains a Tm of at least 42 C." (col 5, line 3).

Nowhere does the Anderson reference teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state, thereby generating a nucleic acid fusion molecule; and **selecting a nucleic acid molecule that encodes a polypeptide wherein the state of the polypeptide encoded by the acceptor nucleic acid is coupled to the state of the polypeptide encoded by the insertion nucleic acid, or the state of the polypeptide encoded by the insertion nucleic acid is coupled to the state of the polypeptide encoded by the acceptor nucleic acid.**

The Anderson reference merely describes GFP fusions that are useful in methods of detection, and in particular with random peptide libraries.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 102 (b)

The Examiner has rejected claims 1 – 5, 7, 8 and 14 under 35 USC 102(b) as being anticipated by Manoil et al. (J of Bacteriology vol. 172, No. 2 p.515 - 518). Applicants respectfully traverse the rejection.

The claims were set forth above.

The Examiner argues that "(t)he insertion of Thpho A into a gene (transposon insertion) is random and the fusion gene encoding hybrid proteins with alkaline

phosphatase activity are detected as blue colonies on media containing the alkaline phosphatase indicator dye." (Office Action, p.7). The Examiner argues that "(g)eneration of the hybrid proteins constitutes insertion of an insertion sequence and said insertion couples the state of the insertion sequence to the state of the acceptor sequence (and) the resulting hybrid protein or gene encoding said hybrid protein is a new state." (Office Action, p.7). Applicants disagree.

Nowhere does the Manoil reference teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, **wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

The Manoil reference is directed to alkaline phosphate fusions that are used as sensors of subcellular location. Manoil describe TnphoA transposons that are used to generate gene fusions coding for hybrid proteins. Manoil et al. describe that "the basis of the alkaline phosphatase fusion approach is that finding the activity of the enzyme response differently to different environments (and) the activity of the fusion protein gives evidence as to its location." (p.517). Manoil et al. are using alkaline phosphatase as a reporter to that varies it function depending on environment.

Accordingly, Applicants respectfully request that the rejection be withdrawn

The Examiner has rejected claims 1 – 5, 7, 8 and 14 under 35 USC 102(b) as being anticipated by Mountford et al. (TIG, Vol 11, No.5, p. 179 - 184). Applicants respectfully traverse the rejection.

The claims were set forth above.

The Examiner argues that the Mountford reference "teaches gene trapping for identifying developmentaly regulated genes based on the random integration of a reporter into chromosomal transcription units." (Office Action, p.8). The Examiner contends that "(t)he gene trap vector is an insertion sequence and the chromosomal transcription units are acceptor sequences...(t)he resulting fusion molecule is a new state." (Office Action, p.8). The Examiner argues that "(a) fusion protein can respond to

a stimulant or inhibitor, therefore, any fusion protein is a modulatable molecule." (Office Action, p.8).

The Mountford reference is directed to the use of IRES elements in transgenic expression constructs to create polyfunctional RNAs. An IRES is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA (mRNA) sequence. Mountford describe various uses of IRES elements or IRES-selectable marker cassettes, for example in coexpression of counter selectable markers (p. 180), in cloning vectors (expression cloning, two-hybrid cloning) and in transgene expression vectors (p.181) and in gene targeting vectors (p.182).

Nowhere does the Mountford reference teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, **wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 102 (e)

The Examiner has rejected claims 1 – 5, 7, 8 and 14 under 35 USC 102(e) as being anticipated by Ong et al. (US Patent No. 6,687,035). Applicants respectfully traverse the rejection.

The claims were set forth above.

Nowhere does the Ong reference teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, **wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

Accordingly, Applicants respectfully request that the rejection be withdrawn

Claim Rejections- 35 U.S.C. § 103(a)

Claims 1 and 45 - 47 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Anderson et al. (as above), in view of Norris, 2006 (US Patent No. 7,135,176). Applicants respectfully traverse the rejection.

Claim 1 was set forth above.

The Anderson et al. reference fails to teach or suggest all the elements of the instant invention. In particular, nowhere does the Anderson reference teach **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, **wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule.

The Norris reference does not cure the defect of the Anderson reference. Nowhere in the Norris reference is there teaching or suggestion of **assembling a modulatable molecule**, comprising inserting randomly an insertion nucleic acid sequence into an acceptor nucleic acid sequence, **wherein the insertion nucleic acid sequence and the acceptor nucleic acid sequence each encode a polypeptide that comprises a state**, thereby generating a nucleic acid fusion molecule. Therefore, the teachings of the cited art, when combined, do not result in the claimed invention.

Accordingly, Applicants request that the rejection be withdrawn.

CONCLUSIONS

For the reasons provided, Applicant submits that all claims are allowable as written and respectfully requests early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

The Director is hereby authorized to charge any credits or deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to Deposit Account No. 04-1105.

Dated: May 8, 2009

Respectfully submitted,

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